

## The Golgi Bodies of a Coccidian.

By

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With Plate 21.

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FOR many months we have been collecting material of the protozoan fauna of the gut of *Lithobius forficatus*. Our object was to study the Golgi apparatus, if such were present, and to examine also the behaviour of the mitochondria. We chose the form *Adelea* because we believed that its life-history was well known, and that there would be no special difficulty in identifying the stages. In this we have been disappointed—we found that Siedlecki and other workers have left the matter in a confused state: we have been obliged to spend many months trying to piece together the various parts of the life-history: at present we are not sure of any of the stages except those described in this paper.

Mr. Dobell (in *literis*) has answered some of our questions as well as he was able considering that his own paper on the subject of *Adelea* was written many years ago at the beginning of his investigations on the Protozoa. In our first material nothing except *Adelea* seemed to be present; but subsequently we found to our regret that the Coccidian (*Eimeria schubergi*?) stages were present side by side with those of *Adelea*. Minchin, in his 'Introduction to the Study of the Protozoa', has brought out the fact that not only has there been a confusion as to the stages in the life-history of many Coccidia, but that in the case of *Adelea*, *Eimeria*, and *Barroussia*, there has been even a confusion of species described under the one name. That this confusion is not difficult to bring about can best be understood by trying to identify the various species and stages; we ourselves have been much puzzled in the endeavour

to identify Siedlecki's stages of macro- and micro-schizonts. We have no wish to enter into a purely protozoological field, but later we shall have to come to some decision ; at present we do not find any clear evidence for the macro- and micro-schizonts of Siedlecki, but wish to leave the matter open. We feel sure, however, that the stages we definitely identify in the present paper are schizonts and gametocytes. No sporogony stages are described.

Several special methods, which show the Golgi apparatus of the metazoan cell, have been used by us. In our material they demonstrated a hitherto undescribed structure in *Adelea*.

Our reasons for identifying this intra-cellular structure as the Golgi apparatus are as follows :

1. Its staining and fixing reactions are identical with those of the Golgi bodies of the metazoa.
2. It occupies an excentric juxta-nuclear position and spreads out in the cell cytoplasm, as does the Golgi apparatus in many metazoan cells, e. g. the egg, and the nerve-cell.
3. It consists mostly of the very characteristically shaped crescents and beads, known, in the case of the metazoan cell, as dictyosomes.
4. As in the metazoan cell, these protozoan dictyosomes can be found dividing by themselves in the ground cytoplasm.

An account of the details of our technique and of our reasons for making the statement in paragraph 1, above, will be given in a later paper.

#### SCHIZOGONY.

The merozoite and sporozoite of certain *Coccidia* are said to be much alike: small nuclear differences have been described: in *Eimeria schubergi* the nucleus of the merozoite is said to have a distinct karyosome, absent in that of the sporozoite. On this basis the stage drawn in Pl. 21, fig. 5, would be a merozoite—it has a karyosome; at the present stage we prefer to call the latter a nucleolus (*n*). Such small crescentic coccidians occur commonly in areas of the *Lithobius* gut, where the asexual multiplication occurs. They are found

among the cells of the gut, intra- and inter-cellular in position, and often in the lumen.

In such stages one can impregnate a ring-like structure, just near the nucleus, in exactly the same position, and of the same general appearance as the Golgi bodies of many metazoan cells. In Pl. 21, fig. 2, is drawn freehand at a very high magnification, a number of these structures we now identify as the Golgi apparatus; the latter consists, as in sponge, coelenterate, and many other cells, of dictyosomes (or Golgi crescents and bent rods) lying upon the surface of a thickened protoplasmic zone or centrosphere. Whether or not a centrosome exists in *Adelea*, there is certainly a darker (denser) zone associated with the sickle-shaped dictyosomes.

As the merozoite (sporozoite) grows and becomes definitely a trophozoite, the Golgi apparatus develops into an important part of the cell: in Pl. 21, fig. 11, *c*, is the Golgi apparatus of a growing coccidian; the apparatus is seen to consist of a number of bent rod-shaped structures, and it is produced by the growth and fragmentation of the original Golgi fragment of the younger cell; the nucleolus, hitherto excentric, becomes more centrally disposed in the nucleus.

In Pl. 21, figs. 12 and 13, the Golgi apparatus is seen to have fragmented partly and the separate dictyosomes are irregularly scattered, though a main aggregation is found at *c*, in a juxta-nuclear position.

Such cells proceed to division; in Pl. 21, fig. 14, is a stage with four nuclei (two shown), and the Golgi bodies had been gathered into four rough groups, one around each nucleus (*x*). The Golgi bodies at this stage were spherical, crescentic, or granular; the next stage is shown in Pl. 21, figs. 16 and 17, where each one of the many nuclei had near it, its own quota of Golgi beads, crescents, and granules. In Pl. 21, fig. 16, the apparatus consisted of closely associated crescents, in fig. 17, of much coarser rings and crescents. Such stages are followed by ones showing the formation of cell-walls, and separation of the merozoites and their subsequent scattering and growth again.

There were from 20 to 30 nuclei in the last stages of division:

no centrosomes could be identified. Among our material one finds all stages from cells like that in Pl. 21, figs. 16, 17, to corps en barillet stages, one of which is depicted in Pl. 21, fig. 18. This is a group of agametes or daughter individuals produced by division of a trophozoite, and now ready to break away into its component parts. Each merozoite has a nucleus with an excentric nucleolus (*n*) which is always turned away from the Golgi apparatus (*g*): no exception to this rule has been found to occur. The Golgi apparatus is either formed of several little crescents together making a little granule, or it is elongate.

In Pl. 21, fig. 20, is a large trophozoite with a completely scattered Golgi apparatus (*g*), which may be seen to lie among other granules (*γ*) whose nature we do not care to examine in this paper. Whether this is an individual of *Eimeria* or *Adelea* we are not sure. Many trophozoites of the *Lithobius* parasites have completely scattered Golgi bodies.

Occasionally one finds schizonts in which some body like the Golgi apparatus (Pl. 21, fig. 15, *gx*) lies in the centre of the cell, and appears to be taking no part in dictyokinesis. This is rare.

#### ♀ AND ♂ (GAMETOCYTES).

In Pl. 21, figs. 19 and 21, are two cells which we can positively identify as ♀ gametocytes—the ♂ gametocyte rests upon them. The Golgi apparatus of the ♀ gametocyte is much like that of the trophozoites already described in Pl. 21, figs. 11, 12, and 13: in some cases it is much more scattered. In the ♂ gametocyte we never found at this association stage a juxta-nuclear and discrete apparatus as in the merozoites in Pl. 21, fig. 18. Even in preparations where the Golgi apparatus of the ♀ gametocyte was beautifully marked as clear black rings and crescents on a yellowish or grey background, the ♂ gametocyte was found only to contain a few black granules (*gx*) generally stuck in its periphery and of doubtful nature. While we cannot positively identify a Golgi apparatus in the ♂ gametocyte at this stage, the small granules which are present, and which might represent

the Golgi bodies, are much fewer and smaller than those found in the ♀ gametocyte.

Examination of a large number of cells which we believe to be ♂ gametocytes before association has enabled us, we think, to throw some light on this matter: in Pl. 21, figs. 1, 3, 4, 6, and 9, are what we believe to be ♂ gametocytes. In them the apparatus is rarely juxta-nuclear, but has fragmented, and its elements seem to have passed to the periphery and are struck beneath, or on, the cell-wall. We have found many ♂ gametocytes in association, with no signs of any Golgi bodies, except a number of these peripheral black granules. We believe that in the ♂ gametocyte the Golgi apparatus is mainly extruded or absorbed. We doubt very much if it takes any part in fertilization. In Pl. 21, fig. 9, is a ♂ gametocyte prepared in the same manner as the cell in Pl. 21, fig. 20. In Pl. 21, figs. 7 and 8, are two cells, which may be intermediates between ♂ and ♀ gametocytes—such intermediates often occur in the metazoa: in Pl. 21, fig. 7, there is certainly an apparatus at *g*, the nature of the granules at *gx* is more doubtful, but in both figures there are elements which seem to be passing to the periphery.

The ♂ gametocyte often stains so darkly that it is difficult to make out much of its structure. In Pl. 21, fig. 10, is an example of a ♂ gametocyte containing a large granule which did not appear to be taking any part in the activity of the cell in which it lay: there were no clear dietyosomes.

#### DISCUSSION.

In this paper we have described what we consider to be the Golgi apparatus of the protozoan *Adelea*. This Golgi apparatus is dissolved away by the same fluids, preserved by the same reagents, and stained by the same methods as the Golgi apparatus of the metazoan cell.

During growth, the coccidian Golgi apparatus, like that of the metazoan egg, spreads out through the ground cytoplasm in the form of curved banana-shaped rods or dietyosomes. So far as we could observe, the Golgi apparatus of *Adelea*

takes no part in the formation of the yolky bodies which are to be found in both trophozoites and gametocyte.

In the asexual multiplication phase where cell-division takes place, the Golgi apparatus behaves as is usual in metazoan cells: it becomes sorted out into subequal groups around each dividing nucleus, and each ultimate daughter nucleus has gathered near it a part of the original apparatus. We have therefore established that a true dictyokinesis takes place in the protozoan *Adelea*.

The Golgi apparatus is found in every schizont and merozoite we have examined, and strangely enough it always lies oriented in a special manner with reference to the excentric nucleolus (karyosome) of the oval nucleus: the Golgi bodies lie always away from the nucleolus. The exact significance of this we do not know: it means possibly that this nucleolus does not contain the body which during division is responsible for shepherding the Golgi elements into groups around the syntyical nuclei (Pl. 21, figs. 16, 17). Whether a true centrosome, either intra- or extra-nuclear is present, we are unable to say: previous workers are mainly against the view that an extra-nuclear centrosome occurs in *Adelea*. Suffice to say at present that within, or near, the coccidian nucleus is some body with the power of attracting the dictyosomes, as occurs in the case of the metazoan centrosome.

The interesting period of conjugation of the gametocytes and of fertilization provided us with no facts worth recording at length. We found not a jot or tittle of evidence for the view that the Golgi apparatus of the male takes part in the process of fertilization: this period is undoubtedly the most difficult to study, and we are not at present satisfied with our material of these stages.

So far as the senior writer is concerned, this examination of the Golgi bodies of a coccidian has been disappointing from the point of view of the phylogenetic origin of the Golgi apparatus; we have found a typically metazoan Golgi apparatus, which acts just like that in the metazoan egg, during oogenesis, and which, as in most cases of metazoan fertilization,



takes no part in the process. We have an apparatus in the coccidian which acts normally at dictyokinesis. It is evident that in this protozoon we have the typical apparatus already formed and established.

The search for a primitive apparatus must be carried out among forms other than the Sporozoa, as Hirschler's and our work amply shows.

The Golgi bodies probably arose in connexion with the terminal bead of the flagellum of some primitive flagellate. The outer layer of the bead might have been differentiated to form a lipid store-house or elaborator of the energy-yielding materials necessary for the nutrition of the locomotor organ. From its primitive position in the metazoan cell, always associated at some time with the centrosome-centrosphere complex, we cannot but believe that in the early history of the cell the Golgi apparatus and the centrosome were evolved side by side, or the apparatus from the centrosphere, in some way. This speculation can only be tested when further evidence is produced. An important field, altogether neglected hitherto, is opened up to protozoologists. The latter more than any of their fellow biologists are interested in the architecture of the organisms which they study, and they should attack the problem with the special methods explained elsewhere (5).

#### SUMMARY.

1. There is a true Golgi apparatus in the *Coccidia* (Pl. 21, figs. 11, 12, 16, 17, 18, 21).

2. It consists of separate dictyosomes or crescentic rods, with the power of fission as in metazoa (Pl. 21, figs. 2, 12, 17).

3. During growth the excentric Golgi apparatus (Pl. 21, fig. 5) becomes larger and tends to spread out in the cell (Pl. 21, figs. 12, 13).

4. During division of the schizont the Golgi elements are attracted into subequal groups of dictyosomes and granules around each nucleus, as happens in most metazoan cell-visions.

5. No centrosome was identified—the Golgi elements are probably attracted by some other body in the nucleus.

6. Each daughter schizont receives a part of the Golgi apparatus of the mother cell.

7. The peculiar nucleolus (or karyosome) of the merozoite (*corps en barillet* stage) always lies at one end of the nucleus. The Golgi apparatus always takes up its position outside at the other end (Pl. 21, fig. 18).

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#### DESCRIPTION OF PLATE 21.

##### LETTERING.

g, Golgi apparatus. gx, bodies possibly either Golgi apparatus modified or derived from the Golgi apparatus. n, nucleus. n, nucleolus (karyosome). y, yolk-like bodies.

Figs. 1, 3, 4, and 10.—Male gametocytes not containing a normal Golgi apparatus. The granules gx, impregnate like the Golgi apparatus, but resemble them neither in position nor morphology.

Fig. 2.—The Golgi apparatus of a number of merozoites drawn free-hand.

Fig. 5.—Young trophozoite just after separation of merozoites.

Fig. 6.—Male gametocyte containing an excentric juxta-nuclear Golgi apparatus, and also numbers of the granules gx, one near the Golgi apparatus.

Figs. 7 and 8.—Cells too large to be normal male gametocytes, but showing what seems to be the degeneration of the Golgi apparatus.

Fig. 9.—Shows yolk-like bodies in male gametocyte.

Figs. 11, 12, 13.—Trophozoite (schizont) showing Golgi apparatus.



Figs. 14, 16, 17.—Stages in schizogony showing dictyokinesis of Golgi elements. In fig. 17 the nuclei are not shown, all the dark rings being Golgi bodies.

Fig. 15.—Four-nuclear stage of schizogony showing the Golgi apparatus (gx) apparently abnormally situated in the centre of the cell and taking no part in division.

Fig. 18.—‘Corps en barillet’ stage showing the merozoites, each with a pale nucleus in which at one end lies the nucleolus, *n* (karyosome); at the opposite pole, outside the nuclear membrane, is the Golgi apparatus.

Figs. 19, 21.—Association of gametocytes: in the  $\sigma^7$  the granules gx were the only bodies which impregnated by Golgi-apparatus methods.

Fig. 20.—Coccidian trophozoite with scattered Golgi elements, and much ‘yolk’, x: the cytocyst lies around the cell.